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# Hematological Effects of Atypical and Cameroon β-Globin Gene Haplotypes in Adult Sickle Cell Anemia

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**To examine the effects of unusual or atypical β-globin gene cluster haplotypes on the hematological features and Hb F levels of sickle cell anemia, we studied African Americans who had an atypical or Cameroon haplotype chromosome in association with a typical haplotype. We identified over 20 atypical haplotypes. The distribution of 5′ subhaplotypes of the atypical chromosomes mirrored the distribution of common haplotypes in African Americans with sickle cell anemia. Neither 5′ nor 3′ subhaplotypes of the atypical chromosomes affected Hb F levels, packed cell volume, or mean corpuscular volume in individuals with a Benin chromosome. That the 5′ subhaplotype is unaffected might be a consequence of the small numbers of Senegal 5′ subhaplotypes in our sample, the need for linkage of both 5′ and 3′ subhaplotypes of any haplotype for an effect on Hb F to be present, or the likelihood that a normal β-globin gene contributed the 5′ subhaplotypes of some atypical haplotypes. Am. J. Hematol. 59:121–126, 1998.**



**Key words: fetal hemoglobin; beta globin gene; sickle hemoglobin; globin gene**

## INTRODUCTION

β-Globin gene cluster haplotypes are defined by restriction fragment length polymorphisms (RFLPs) in and around the β-globin–like genes [1–3]. Three common haplotypes are recognized in most patients with sickle cell anemia: Benin; Bantu (also known as Central African Republic (CAR); and Senegal types [4–6]. One less frequent haplotype, the Cameroon, is restricted in Africa, to the Eton ethnic group [7]. These haplotypes have some use as genetic markers of the phenotypic heterogeneity of patients with sickle cell anemia [6,8–18]. Linked to a haplotype are genetic elements likely to partake in the modulation of fetal hemoglobin (Hb F) levels in sickle cell anemia [6,10–12,15,18–24].

In two large studies of sickle cell anemia in which the β-globin gene haplotype was determined, about 20% of the patients had either an atypical or a Cameroon haplotype chromosome [18,24]. Atypical Hb S haplotypes in African Americans are a result of genetic admixture. Africans with sickle cell anemia are usually homozygous for the β-globin gene cluster haplotype. If the typical haplotype linked to Hb S in a particular region of Africa was also common in $\beta^A$ chromosomes, the recombination events would produce a typical instead of an atypical haplotype. This is apparent in central West Africa where the Benin haplotype is linked nearly 100% to the $\beta^S$ gene and is also linked to the $\beta^A$ gene in 60% of the general central West African population. In the Americas, the $\beta^A$ chromosome originated from throughout Africa and hence the black population lacking the $\beta^S$ gene has a more diverse distribution of haplotypes. Genetic admixture with Caucasian genes—at about 20%–30%—adds to the genetic diversity [25]. These factors explain the ob-

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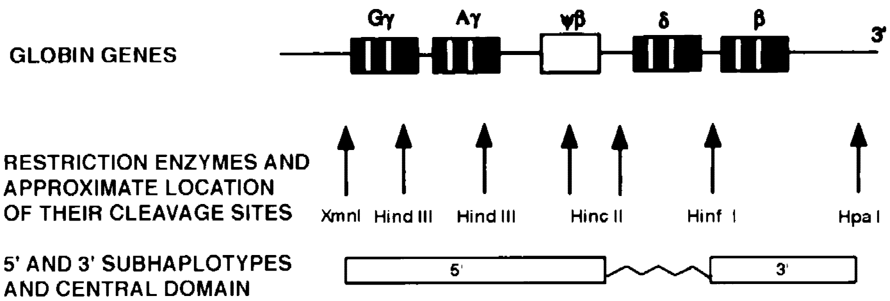


**Fig. 1. The β-like globin gene cluster, showing the restriction endonucleases used to determine the β-globin gene haplotype, the approximate location of the cleavage sites examined (arrows), and the 5′ and 3′ subhaplotypes. These subhaplotypes are connected by a central domain of ~9 kb where recombination takes place.**

servation that in North America, the $\beta^S$ gene is linked to an atypical haplotype more frequently than in any region of Africa. Recombination, accounting for most atypical haplotypes, is common around the ''hot spot'' 5′ to the δ-globin gene [26,27] (Fig. 1).

Haplotypes are associated with *cis*-acting elements that regulate the expression of the γ-globin genes [28,29]. This association is likely to be mediated through regulatory domains whose locations and modes of action are still unclear [28–30]. Since atypical haplotypes arose by recombination, they contain regulatory elements of two different haplotypes. To examine the effects of atypical haplotypes on the hematological features and Hb F level in sickle cell anemia, and to attempt to see whether it was possible to dissociate the effects of regulatory domains linked to the 5′ or 3′ portion of an atypical haplotype, we studied African Americans with sickle cell anemia who had an atypical or a Cameroon haplotype chromosome.

## METHODS

### Patients

Atypical haplotypes reported here were detected in previous studies of sickle cell anemia [11,18,24].

### Hematological Tests

Blood counts were done by routine methods using automated cell counters. Hb F levels were obtained by high performance liquid chromatography (HPLC) [31] or alkali denaturation [32]. There is an excellent correlation of Hb F levels measured by alkali denaturation and HPLC at Hb F levels between 1% and 10%. Above 10% Hb F, alkali denaturation is less accurate and gives values lower than HPLC methods.

### Haplotypes

β-Globin gene haplotypes were done by either of two methods or combinations of these techniques as described previously [33].

### Statistics

Differences among the groups formed for analysis were compared using the students t-test with the Bonferroni correction for multiple samples.

## RESULTS

### Patient Sample

Atypical or Cameroon haplotype chromosomes were present in 112 patients. Their mean age was 30.8 years. No significant age differences existed in any of the groups analyzed.

### Atypical Haplotypes

One hundred fourteen atypical or Cameroon chromosomes were analyzed (two patients were homozygous for atypical chromosomes). Possible crossovers that lead to atypical chromosomes are shown in Table I. Of the 107 typical haplotype chromosomes, 68.2% were Benin, 18.2% were CAR, and 10.9% were Senegal. Three instances existed in which a Cameroon chromosome was associated with an atypical haplotype.

Thirty-three percent (35) of the 5′ subhaplotypes of atypical chromosomes (determined by the *Xmn*I site 5′ to the $^G\gamma$ gene, *Hind*III sites in the $^G\gamma$ and $^A\gamma$ genes, and *Hinc*II sites in the ψβ gene and 5′ to the δ-globin gene) were consistent with a typical sickle cell anemia haplotype. Of these, 55.8% were Benin, 17.6% were CAR, 14.7% were Senegal, and 11.7% were Cameroon. This distribution reflects the prevalence of typical haplotype $\beta^S$ chromosomes in the African American population [10,11,18,34–36]. Sixty-seven percent of 5′ subhaplotypes of atypical chromosomes were derived from $\beta^A$ chromosomes [34]. It was not possible to determine whether these chromosomes were of African or Caucasian origin.

Because of the choice of restriction sites examined, it was possible to determine the 3′ subhaplotype (*Hinf*I site 5′ to the β-globin gene and *Hpa*I site 3′ to the β-globin gene) in only 75% of the atypical chromosomes. These included 16% Benin, 22% CAR, 21% Senegal, and 7% others. Cameroon chromosomes comprised 34% of the unusual haplotypes that we examined.

### Hb F and Hematological Findings

To determine whether there was an effect of the 5′ and 3′ subhaplotype of the atypical haplotype, or of the Cameroon haplotype on Hb F or hematological values, we separated our patients as follows: 1. Typical haplotype

**TABLE I. Potential Crossovers Responsible for Atypical Haplotypes Found in This Study***

| Atypical chromosome | | | | | | | Crossover between typical and $\beta^A$ or $\beta^S$ chromosome |
|---|---|---|---|---|---|---|---|
| | | | | | | | 1–5′ $^G\gamma$ *Xmn*I, 2-$^G\gamma$ *Hind*III, 3-$^A\gamma$ *Hind*III, 4-ψβ *Hinc*II, 5– 3′ ψβ *Hinc*II, 6–5′ β *Hinf*I, 7–3′ β *Hpa*I[a] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| − | − | − | − | + | − | + | − − − − + − − (11, Ben) × − + − − − − − + (CAR) |
| − | − | − | − | − | − | − | − − − − − + + (12) × − − − − − + − − (Ben) |
| − | − | − | − | − | − | + | − − − − − + + (12) × − + − − − − + (CAR) |
| − | + | + | − | + | − | + | − − + + − + + + (Cam) × − + − − − − − + (CAR) |
| − | + | − | − | + | + | + | − + − − + + + (13) × − − − + + + (Sen, Cam) |
| − | − | − | − | + | + | − | − − − − + + + (11) × − − − − − + − − (Ben) |
| − | − | − | − | − | + | + | − − − − − + + (12) × − − − − − − − + (CAR, Sen, Cam) |
| − | − | − | − | + | + | + | − − − − + − − (11, Ben) × − − − − − − + + (Sen, Cam) |
| − | + | − | − | − | − | − | − + − − − − + (CAR) × − − − − − + − − (Ben) |
| − | + | − | − | + | − | − | − + − − + + + (13) × − − − − − + − − (Ben) |
| − | + | − | − | − | + | + | − + − − − − + (CAR) × − − − − − − + + (Sen, Cam) |
| − | + | − | − | + | − | + | − + − − + + + (13) × − + − − − − + (CAR) |
| + | + | − | − | + | − | − | + + − + + + + (Sen) × − − − − − + − − (Ben) |
| + | + | − | + | + | − | + | + + − + + + + (Sen) × − + − − − − − + (CAR) |
| − | + | − | − | + | + | − | − + − − + + + (13) × − − − − − + − − (Ben) |
| − | − | − | − | − | + | − | − − − − − + + (12) × − − − − − + − − (Ben) |
| − | − | + | − | + | − | − | ? |
| − | + | + | − | − | + | + | ? |
| + | + | − | + | − | + | + | ? |

*Ben, Benin; CAR, Central African Republic; Cam, Cameroon; Sen, Senegal.

[a]The numbers 1 through 7 precede each genomic site and the restriction enzyme used. A minus sign (−) indicates absence of cleavage at the cognate restriction endonuclease site whereas a plus (+) indicates the presence of enzyme cleavage. In 5′ to 3′ order, the sites around and within the ϵ-$^G\gamma$-$^A\gamma$-ψβ-δ-β-globin gene complex examined in the majority, but not all of the patients, were: *Xmn*I 5′ to $^G\gamma$, *Hind*III within $^G\gamma$ and $^A\gamma$, *Hinc*II within and 3′ to ψβ, *Hinf*I 5′ to β, and *Hpa*I 3′ to β. In some patients, the 3′ subhaplotype was determined by examining the *Ava*I site within the β-globin gene and the *Bam*HI site 3′ to the β-globin gene instead of the *Hinf*I site 5′ to the β-globin gene and *Hpa*I site 3′ to the β-globin gene. The column ‘‘crossover between,’’ denotes some of the possible crossovers between typical Hb S haplotype chromosomes or $\beta^A$-globin gene haplotype chromosomes and typical Hb S haplotypes that generated the atypical haplotypes. The numbers assigned to the certain haplotypes involved in putative crossovers (in parentheses) are from Antonarakis et al. [3] and usually represent chromosomes in African Americans that are associated with the $\beta^A$-globin gene.

chromosomes with any atypical haplotype (Table II); 2. A Benin haplotype with 5′ or 3′ subhaplotypes of atypical chromosomes (Table III); and 3. Cameroon haplotype chromosomes with typical Hb S haplotypes (Table IV). Hb F levels and other hematological measurements differed little among these diverse groups. There appeared to be little effect on Hb F, packed cell volume (PCV), or mean corpuscular volume (MCV) when the Cameroon haplotype was present with the Benin, CAR, or Senegal haplotypes (Table IV). Mean Hb F level in patients with atypical chromosomes that had the CAR 5′ subhaplotype, regardless of whether it was present with a typical Benin, CAR, or Senegal haplotype chromosome, was similar to Hb F in individuals with a Benin 5′ subhaplotype.

Seventeen patients had Hb F ⩾ 10% (mean 14.4 ± 4.0%) and 14 patients had Hb F ⩽ 2% (mean, 1.1 ± 0.1%). In individuals in which one chromosome was a Benin type, 11 patients had Hb F ⩾ 10% and six had Hb F ⩽ 2%. Of the patients with high Hb F, there was one atypical chromosome with a 5′ Senegal subhaplotype and one with a Cameroon haplotype. Two individuals with low Hb F levels had CAR 3′ subhaplotypes.

The atypical chromosome in 13 individuals (10 with a Benin haplotype, one with a CAR haplotype and two with a Senegal haplotype) had a Senegal or Cameroon 3′ subhaplotype. Their Hb F was 9.01 ± 6.2%. Hb F was 9.5 ± 7.1% in the 10 patients with the Benin chromosome. In four patients with a Senegal 5′ subhaplotype, all with a typical Benin chromosome, Hb F was 7.2 ± 2.4% (Table III). The differences in Hb F in these groups were not significant.

**TABLE II. Hematological Effects of Combined Heterozygosity for Typical and Atypical Haplotype Chromosomes in Sickle Cell Anemia***

| | n | Hb F (%) | PCV | MCV (fl) |
|---|---|---|---|---|
| Benin | 75 | 5.9 ± 4.7 | 24.4 ± 4.6 | 94.1 ± 9.4 |
| CAR | 20 | 5.7 ± 5.0 | 24.1 ± 3.9 | 91.6 ± 10.4 |
| Senegal | 12 | 5.4 ± 3.8 | 24.7 ± 3.7 | 87.1 ± 13.0 |

*Hb F, fetal hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; CAR, Central African Republic.
Hb F levels, PCV, and MCV when a typical sickle cell anemia haplotype chromosome (column 1) is associated with any of the atypical haplotype chromosomes shown in Table I. Results are expressed as the mean ± 1 SD. Two individuals were homozygous for atypical haplotypes. In these individuals, one was homozygous for the CAR 5′ subhaplotype, and the other was homozygous for the Benin 5′ subhaplotype.

## DISCUSSION

Atypical haplotypes in sickle cell anemia usually result from recombination between haplotypes common in

**TABLE III. Hematological Effects of the Interactions Between the Benin Haplotype Chromosome and 5′ or 3′ Subhaplotypes of Atypical Chromosomes***

| Atypical | n | Hb F (%) | PCV | MCV (fl) |
|---|---|---|---|---|
| 5′ Benin | 12 | 6.9 ± 5.4 | 25.2 ± 4.9 | 93.5 ± 9.4 |
| 5′ Other | 37 | 5.6 ± 3.5 | 24.1 ± 5.1 | 93.4 ± 10.1 |
| 5′ Senegal | 4 | 7.2 ± 2.4 | 22.8 ± 5.8 | 102.1 ± 9.0 |
| 3′ Benin | 12 | 4.0 ± 2.4 | 24.7 ± 4.8 | 95.0 ± 9.4 |
| 5′ CAR | 12 | 4.8 ± 3.8 | 23.6 ± 4.7 | 95.4 ± 9.7 |
| 3′ Senegal[a] | 10 | 9.5 ± 7.1 | 25.2 ± 5.8 | 91.1 ± 9.2 |

*See Table II. All individuals reported in this table have a typical Benin chromosome and an atypical chromosome in *trans.* The first column (atypical) lists 5′ and 3′ subhaplotypes of the atypical chromosome. ‘‘Other’’ indicates all other 5′ haplotype besides the Benin type.

[a]We were unable to differentiate between Senegal and Cameroon, 3′ subhaplotypes because of the restriction enzyme sites that were chosen for the original assignments of haplotype. The diversity of 5′ and 3′ subhaplotype among the 19 individuals with a CAR typical haplotype and an atypical haplotype and the few individuals with Senegal and Cameroon chromosomes and atypical haplotypes precluded their analysis.

**TABLE IV. Effect of the Cameroon Chromosome in Haplotype Combined Heterozygotes***

| | n | Hb F | PCV | MCV |
|---|---|---|---|---|
| Typical Haplotype | | | | |
| Benin | 20 | 5.9 ± 4.0 | 24.8 ± 4.0 | 97 ± 8.9 |
| CAR | 7 | 5.2 ± 3.6 | 24.4 ± 5.5 | 92 ± 13.6 |
| Senegal | 3 | 6.2 ± 5.7 | 23.4 ± 4.6 | 93 ± 9.9 |
| All | 30 | 5.8 ± 3.7 | 24.6 ± 5.2 | 93 ± 10.5 |

*See Table II footnote. All patients were combined heterozygotes for a Cameroon haplotype chromosome and one of the typical haplotype chromosomes (column 1).

sickle cell anemia and haplotypes rarely associated with the $\beta^S$-globin gene. RFLPs used to assign a haplotype are present in a 63 kb stretch of DNA subdivided into a 34 kb 5′, 19 kb 3′, and 9 kb central domain (Fig. 1). RFLPs within the 5′ and 3′ sectors are nonrandomly associated, whereas the central region is randomly associated with either 5′ or 3′ domains. In the central domain, 5′ to the δ-globin gene, is a ‘‘hot spot’’ for recombination [3,27]. γ-globin gene expression is influenced by *cis*-acting elements linked to a haplotype and Hb F modulates the phenotype of sickle cell anemia [28,29]. These elements are dispersed throughout the β-globin gene cluster in its locus control region (LCR) [37–39], the 5′ to the γ-globin genes [40], and 5′ to the β-globin gene [41,42]. As most of these elements lay 5′ to the δ-globin gene, they are likely to be linked to the 5′ subhaplotype. We reasoned that by examining the association of 5′ and 3′ subhaplotypes with selected hematological characteristics of sickle cell anemia patients, we might gain further insights into their effects on disease phenotype.

In one report, a sickle cell anemia patient homozygous for the Benin haplotype had 21% Hb F and 65% $^G\gamma$-chains, values more consistent with the Senegal haplotype. A crossover 5′ to the $^G\gamma$ gene was suggested by the presence of Senegal haplotype 5′ HS 2 sequences and the absence of the −158 *Xmn*I restriction site [37]. In studies of larger numbers of patients, these polymorphic regions were always haplotype-linked and additional mutations were not found, suggesting that any variations in these areas, uncoupled from the haplotype, are uncommon mechanisms of Hb F modulation in sickle cell anemia [28,29].

Over 20 atypical haplotypes were identified. This diversity may be explained by the many different genetic backgrounds associated with the β-globin gene in African Americans [3] (Table I). As expected, the distribution of 5′ subhaplotypes mirrored the distribution of haplotypes in African Americans with sickle cell anemia [10,11,18,34–36]. Neither 5′ nor 3′ subhaplotypes affected Hb F levels, PCV, or MCV. In African Americans, Senegal and CAR are less common than Benin haplotypes, so fewer atypical chromosomes bearing Senegal or CAR subhaplotypes were found. Carriers of a CAR haplotype have lower Hb F levels and individuals with a Senegal haplotype have higher Hb F levels [6,11,18,24].

Our failure to find lower Hb F concentrations with 5′ or 3′ CAR subhaplotypes or higher Hb F levels associated with Senegal subhaplotypes—these haplotypes are most likely to contain elements responsible for higher or lower Hb F levels—may be a result of the small numbers of patients in these subgroups of our patient sample. Alternatively, the possibility exists that the dissociation of 5′ and 3′ regulatory elements nullifies the haplotype-like effect on γ-globin gene expression in *cis.* In our analysis we have not considered modulation of Hb F by putative genetic elements not linked to the β-globin gene cluster [43,44]. In sickle cell anemia, one of these elements, the F-cell production locus, was believed to account for about half of the variation in Hb F levels [15].

One important additional feature of genetic recombination deserves mention. When a 5′ subhaplotype, including the LCR, becomes linked to the $\beta^S$-globin gene by recombination, there is no assurance that polymorphisms in the LCR—or any other region of the chromosome 5′ to the site of recombination—will be the same as that linked to the $\beta^S$-globin gene in the original haplotype. For example, a 5′ Benin subhaplotype from a $\beta^A$ chromosome may contain polymorphisms distinct from those of the 5′ Benin subhaplotype linked to the $\beta^S$-globin gene. The $\beta^S$-globin gene has been linked to the 5′ Benin haplotype for only 2,000–3,000 years, the approximate time of origin of the Hb S gene, whereas 5′ subhaplotypes linked to the $\beta^A$-globin gene have evolved for a considerably longer interval. This would allow for many different polymorphisms to be associated with the LCR—and other 5′ regions—of the $\beta^A$ chromosome compared to the $\beta^S$ chromosome where the sequence of

the LCR is relatively monotonous. In some atypical haplotypes, the 5′ subhaplotype is likely to be of Caucasian origin, further contributing to genetic diversity and uncoupling the $\beta^S$-globin gene from the influence of genetic elements that may have co-evolved to maintain high Hb F levels.

Whereas atypical or Cameroon haplotypes appear to have little effect on the hematological findings or Hb F level in sickle cell anemia, small differences may require a much larger patient sample to detect.

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